Brief Description of the Tables

Table 1 summarizes the strategy for purifying a thiaminase, using *Naegleria* thiaminase as an example.

Table 2 presents a sample of the evidence that *Naegleria* thiaminase expressed in *E. coli* induces apoptosis, and that its ability to induce apoptosis depends on the thiaminase activity rather than any other feature of the protein.

Brief Description of the Drawings

Figure 1 is a graph showing that the *Naegleria* agent (Nex) depletes thiamin from the growth medium. Rat glioma C6 cells were grown in Medium 199 with 10% fetal bovine serum. The amount of thiamin in the medium was measured using the thiochrome method ((Wyatt et al., 1989)), in two independent experiments (open symbols and filled symbols) with cultures treated with the *Naegleria* agent at dilutions of 1 □ 10^{-4} and 1 □ 10^{-5}.

Figure 2 shows that addition of thiamin can reverse progress toward apoptosis. Cells were plated with 2 □ 10^{-5} Nex. Thiamin, at 3 μM, was added to cultures at 12–24 hour intervals. In this experiment the latent period was 4.5 days.

Figure 3 shows the staining of a polyacrylamide gel of partially purified *Naegleria* agent using the diazo reagent. The clear area indicates the band that contains thiaminase.

Figure 4 shows the 3414 base sequence of the coding region of *Naegleria* gene TTK; the first segment of this gene (underlined) encodes thiaminase I.

Figure 5 shows the amino acid sequence encoded by *Naegleria* gene TTK.

Figure 6 shows the DNA sequence of the 1068 base segment that encodes the Naegleria thiaminase I, as obtained from *Naegleria* gene TTK. This segment, expressed as pNB1+, encodes catalytically active thiaminase.

Figure 7 shows the 356 amino acid sequence encoded by the *Naegleria* gene segment expressed in pNB1+, along with the DNA codons.

Figure 8 is an alignment comparing the amino acid sequence of *Naegleria* thiaminase I to other homologous sequences, specifically the thiaminase I of *Bacillus* thiaminase I and segments of several transketolases, which we found show limited homology to the encoded sequences of the two sequenced thiaminase I proteins.

days after morphological death of C6 cells ≤0.00006% clonogenic survivors remain. The agent must be present in the culture until close to the time of morphological death. If the agent is removed and rinsed away even 12 hours prior to the end of the latent period, the cells remain healthy, as if never treated. The mode of cell death is apoptosis by all tested criteria, including membrane bubbling, margination of chromatin, cleavage of nuclear DNA to oligonucleosomal fragments, externalization of phosphatidylserine, and production of apoptotic bodies.

The *Naegleria* agent can induce apoptosis in nonproliferating cells, even if the agent is added after the cells have reached stationary phase; under appropriate conditions we have demonstrated that cells can die without re-entering the S phase (DNA synthesis) of the cell cycle. The agent can also induce apoptosis in the absence of protein synthesis, indicating that it action activates the constitutive cell suicide machinery without requiring the translation of new proteins. The ability of the agent to kill is not affected by overexpression of the anti-apoptosis oncogene *bcl-2*. Finally, the agent is similarly effective in killing cells that express wild-type p53 or cells unable to express p53. These features offer great potential advantages for cancer therapy.

The agent acts by depleting thiamin (vitamin B1) from the medium and thus creates a thiamin deficiency, specifically by acting as a thiaminase (Fulton et al. *supra*). The rapid depletion of thiamin from the medium by the *Naegleria* agent is shown in Fig. 1. At a 10^{-4} dilution, thiamin was unmeasurable within 4 hours. The addition of excess thiamin to cell cultures treated with the *Naegleria* agent at any time prior to the first signs of morphological death can prevent the induction of apoptosis (Fig. 2). Adding thiamin is equivalent to replacing the agent-treated growth medium with fresh medium to reverse the effect of treatment. The addition of thiamin acts as an "antidote" for the apoptosis-inducing activity of the *Naegleria* agent. This ability to reverse the effect of the agent is an unusual and powerful asset to therapeutic use. If, unexpectedly, targeted therapy using thiaminase got out of control (e.g., if unacceptable physical effects were observed), an antidote would always be available in case untargeted cells were affected at an unacceptable level or an excessive overall thiamin deficiency was created in the patient.

Any regime of drug administration, especially those involving conventional chemotherapies, includes the possibilities of drug overdose. Even with targeted